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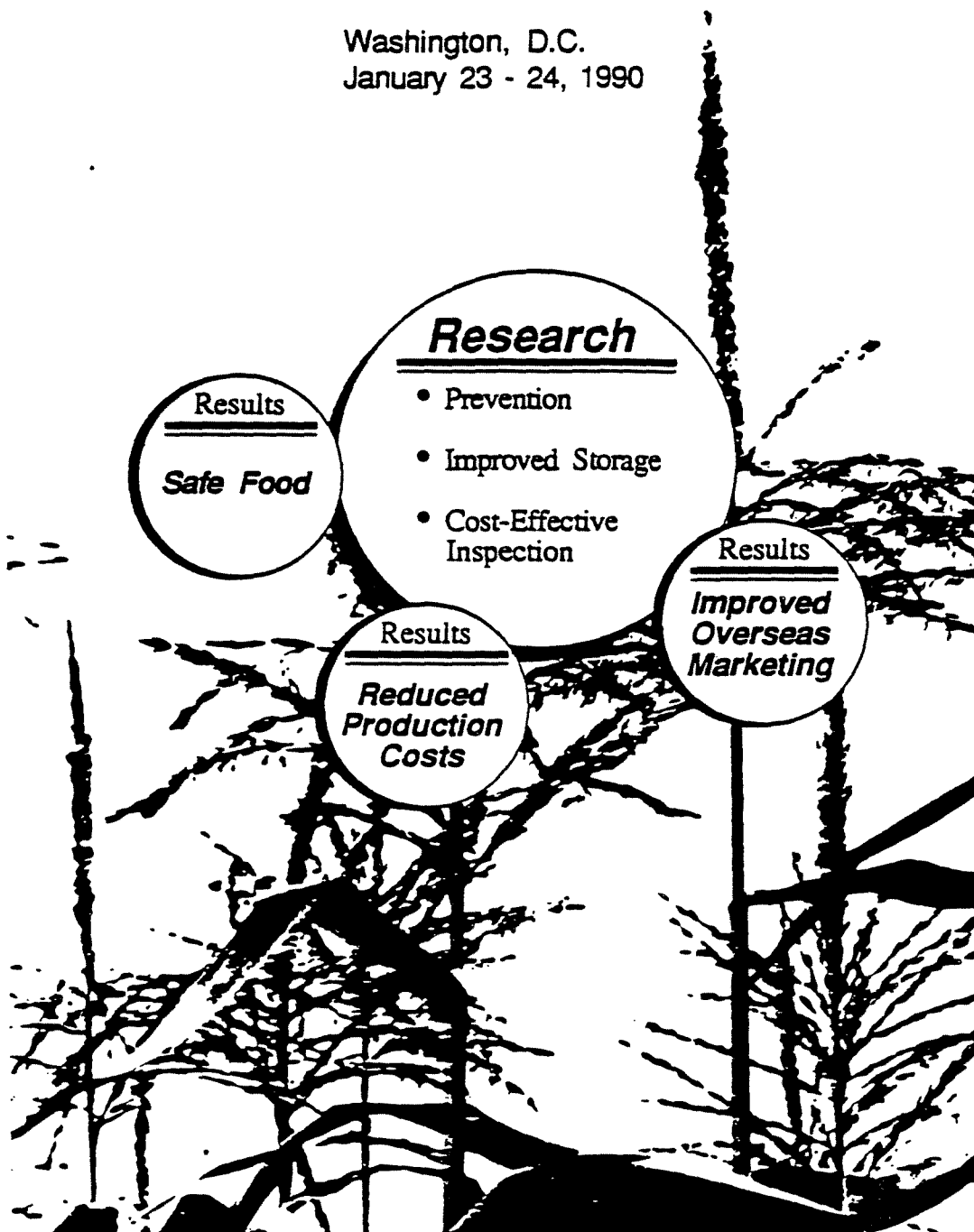
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# A Perspective on Aflatoxin in Field Crops and Animal Food Products in the United States

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## Topic II

### Environmental Factors and Other Stresses Important in the Production of Aflatoxin in Corn

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*Aspergillus flavus* produces yellow-green spores that function in dispersal and as infective inoculum, in addition to long-lived survival structures called sclerotia. Both types of propagules are associated with damaged corn kernels and are dispersed onto the ground during combine-harvesting (Wicklow and Horn, 1984; Wicklow et al., 1984). Sclerotia are able to survive overwintering in Georgia and Illinois (Wicklow, 1987). Upon germination, the sclerotia produce large numbers of yellow-green spores (Fig. 1; Wicklow and Donahue, 1984). Wicklow and Wilson (1986) found that sclerotium germination occurred in corn fields just prior to silking. Shade provided by the corn canopy helps to retain moisture at the soil surface, thus promoting germination. Stack and Pettit (1984) reported germination of buried sclerotia at soil moisture levels slightly below saturation with subsequent fungal colonization of dead roots. Therefore, simple burial of *A. flavus* sclerotia by plowing will not necessarily eliminate this important source of primary inoculum. Soil-inhabiting fungivorous mites disperse spores throughout the soil and carry them through cracks in the peanut fruit (Aucamp, 1969).

We recently contrasted the survival of both sclerotia and spores of *A. flavus* that were buried for 6 months to 3 years (Oct. 1986 - Oct. 1989) in sandy field soil in southern Georgia and central Illinois (D.T. Wicklow & D.M. Wilson, unpublished results). Nearly all of the *A. flavus* spores had disappeared within the first year, while many sclerotia remained viable and produced spores after 3 years. Another soil-inhabiting fungus, *Paecilomyces lilacinus* (Thom) Samson colonized and rotted many of the *A. flavus* sclerotia, thus pointing to a potential means of biological control (Wicklow and Wilson, 1990). Sclerotium survival is poor in heavier soils that become seasonally inundated (Wicklow, 1987). The opportunity to eliminate *A. flavus* inoculum from soil occurs in fields that are seasonally rotated from rice to peanuts as practiced in some areas of Thailand (Wicklow, 1989). Unfortunately, this approach is not feasible in sandy, well-drained soils typical of the Georgia Coastal Plain. An understanding of the dynamics of *A. flavus* populations in soils under cultivation (Angle et al., 1982; Griffin and Garren, 1976; Martynink and Wagner, 1978) is central to the design of agronomic/cultural practices with the objective of reducing levels of *A. flavus* inoculum in those soils.

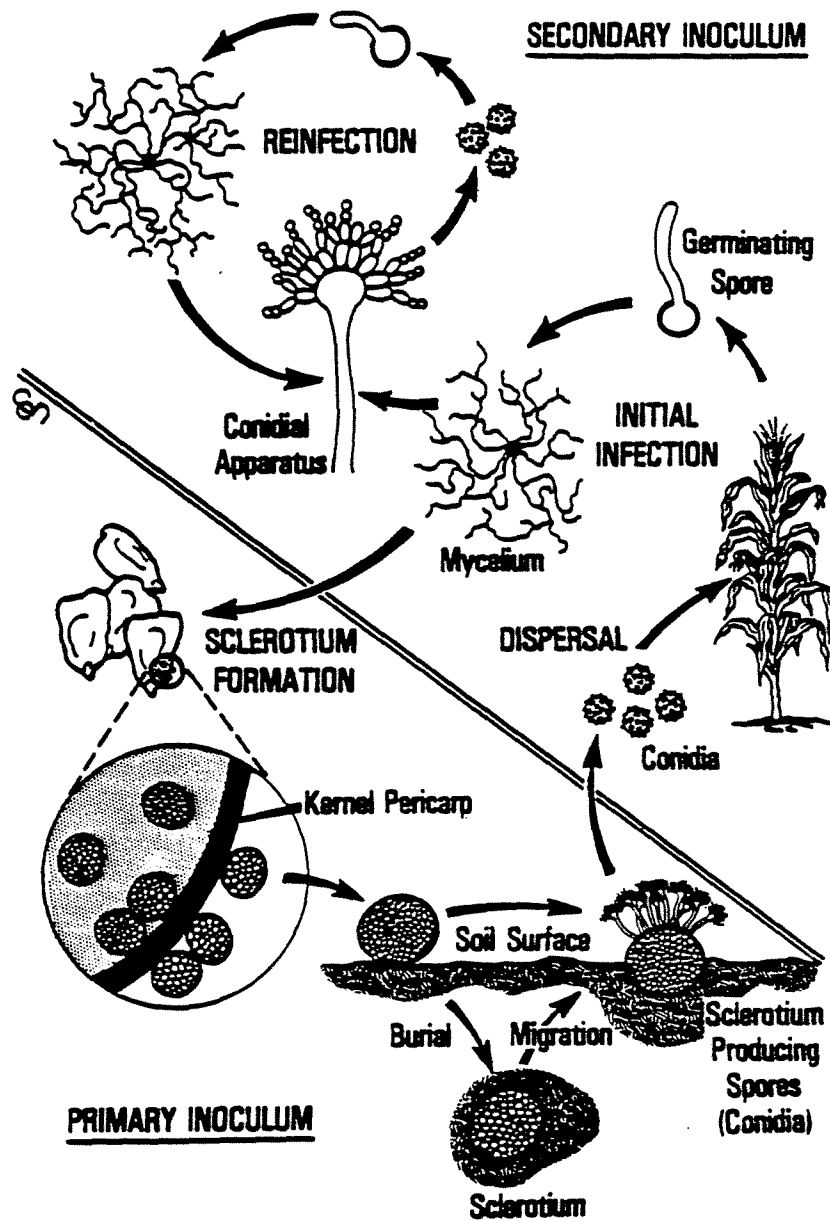


Figure 1.  
Schematic diagram, not to scale, showing the relationship between primary and secondary inoculum in the life cycle of *A. flavus* (Wicklow and Donahue, 1984).

The most widely accepted model of *A. flavus* contamination of corn (Diener et al., 1987) involves three steps: (1) airborne or insect-transmitted spores contaminate corn silks, and the fungus then grows down through the silks into the developing ear; (2) portions of the ear having kernels damaged by insects or birds become rotted by *A. flavus* and contaminated with aflatoxin; (3) cultural factors that stress the corn plant, such as drought, increase susceptibility to fungal infection. Initial attempts to identify insects that might carry *A. flavus* to maize have proven inconclusive (Diener et al., 1987). While many kinds of insects collected from maize ears are reported to carry *A. flavus* (Fennell et al., 1977), collections have typically been made late in the season at a time when secondary inoculum of *A. flavus* is so abundant that insects are more likely to become contaminated. These studies did not identify the initial source(s) of *A. flavus* infective inoculum. A significant association between earworm damage, presence of 'picnic beetles' (Nitidulidae: Coleoptera), and incidence of aflatoxin contamination in corn has been reported in the southern United States (Lillehoj et al., 1975), but the authors did not consider the possibility that picnic beetles were potential vectors of *A. flavus*. Our research shows that these beetles overwinter in and feed on molded crop residues (e.g. lodged corn ears) and carry *A. flavus* spores to ripening corn ears (Fig. 2). The beetles gain entry to the ears through wounds caused by other insects, birds, etc. and are capable of entering some ears on their own (Connell, 1956; Tamaki et al., 1982; Wicklow, 1989). Increased losses in corn from insect damage is attributed to new early maturing hybrids with loose, open husks and quick dry-down attributes. These hybrids have recently been introduced in the southeast (Barry et al., 1986; McMillian, 1987), and offer ready access to picnic beetles ~~entry/entry~~.

When *A. flavus*-contaminated picnic beetles fly to damaged maize ears, they contaminate the wounds with *A. flavus* spores. The damaged kernels become contaminated with substantial quantities of aflatoxin (i.e. to @ 60,000 ppb). The fungus can then spread to infect the adjacent sound kernels (Wicklow et al., 1988). Aflatoxin also accumulates (to @ 4,000 ppb) in many of the 'sound kernels.' It takes only a few of these aflatoxin-contaminated kernels in a grain sample to register a bulk concentration > 20 ppb aflatoxin. Efforts to identify maize genotypes resistant to *A. flavus* kernel rot and aflatoxin contamination have been unsuccessful (Davis et al., 1986). No maize genotype has a complete defense against ear-feeding insects, and no half-eaten kernel is resistant to molding by *A. flavus*.

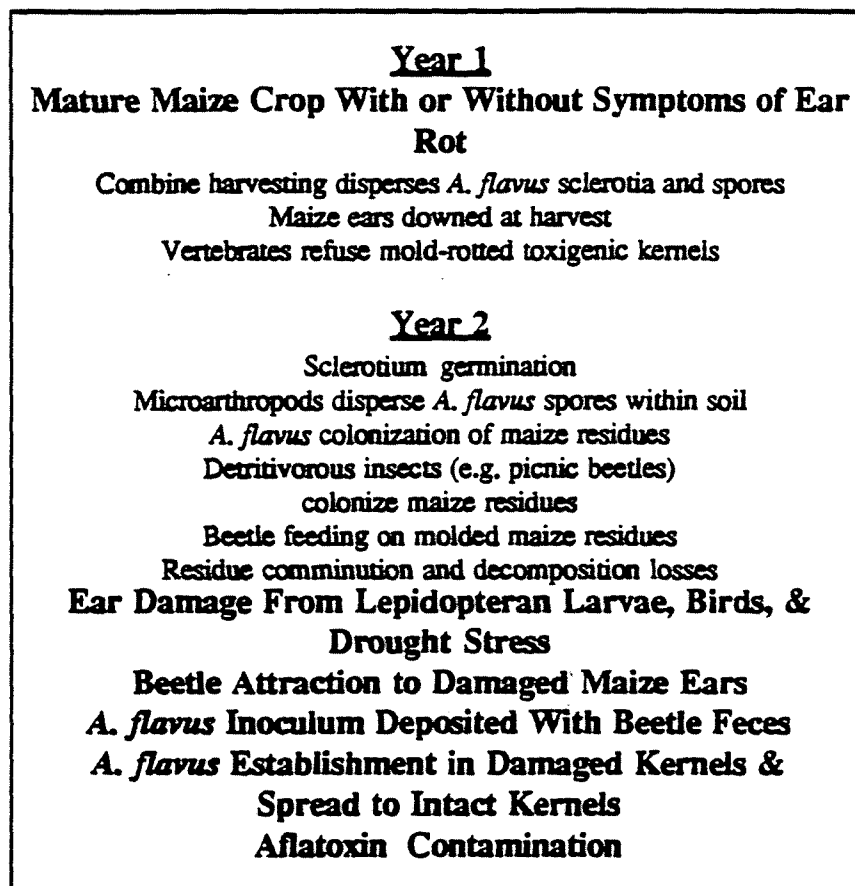


Figure 2.

A simplified conceptual model of how preharvest maize becomes infected with *A. flavus* and contaminated with aflatoxins. Events occurring in the standing maize crop depicted in UPPER CASE Letters, with action on ground indicated by lower case letters (Wicklow, 1989).

Picnic beetle aggregation patterns in corn fields could explain the irregular distribution of *A. flavus* and aflatoxin among damaged ears from the same field. One strategy that has proven effective in controlling picnic beetles, in fig orchards, is the use of bait trapping (Warner, 1960; Warner, 1961). Our discovery that picnic beetles produce aggregation pheromones that can be synthesized (Bartelt et al., 1990), and that plant and fungal volatiles, from decaying host tissues, synergize the attractancy of the beetle aggregation pheromone (Dowd and Bartelt, 1988), should enable us to develop superior traps.

Temperature stress (evening temperatures > 80 C), drought stress, nitrogen stress and crowding of corn plants are all associated with *A. flavus* contamination and aflatoxin formation (Anderson et al., 1975; Davis et al., 1986; Jones et al., 1981b; Smart et al., 1990; Thompson et al., 1980). The cause of this association is believed to be the greater susceptibility of corn plants to fungal invasion when they are stressed (Jones and Duncan, 1981a). Zuber et al. (1983) found that high temperatures, especially during grain-filling stages, were more important than moisture in enhancing the level of aflatoxin. Drought and high temperatures should also promote the proliferation of *A. flavus* populations in and around corn fields, although there is no experimental data showing that, with prolonged drought, corn insects become contaminated more likely with *A. flavus* before flying to the corn.

I have attempted to illustrate how variations in climate, sources of fungal inoculum, potential insect vectors and the response of corn plants to stress can interact in various ways to produce an aflatoxin outbreak. Our integrated disease management program seeks to reduce damage to corn ears from drought/temperature stress and from insects while attempting to eliminate natural reservoirs of *A. flavus* spores and sclerotia. The screening of corn hybrids for resistance to aflatoxin contamination has been disappointing because of the variable amounts of aflatoxin at different geographical locations and from year to year (Davis et al., 1986; Widstrom et al., 1984, 1987). It is difficult to select for drought stress resistance when conditions necessary for its expression cannot be controlled. Side-by-side dryland and irrigated trials (Jensen and Cavaliere, 1987) should be used in testing corn varieties for resistance to insect damage and *A. flavus* infection under drought and temperature stress at a location, where aflatoxin contamination of corn is a recurrent problem. In 1989 we began cooperative research with a major commercial corn seed company, to screen their corn hybrid genotypes for resistance to *A. flavus* infection and aflatoxin contamination of the kernels. We are encouraged by preliminary results showing that our screening procedure can pair rows of the same hybrid when planted as part of a blind screen.

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